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ASSESSING EXPOSURE TO ENVIRONMENTAL TOBACCO SMOKE: IS IT VALID TO EXTRAPOLATE FROM ACTIVE SMOKING?

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Abstract

This review examines the question of whether exposure to environmental tobacco smoke (ETS) can be assessed by extrapolation from active smoking. General problems associated with assessing exposure to ETS and the pathophysiological consequences are discussed. Among the topics presented are the dynamic chemical and physical characteristics of ETS and exposure assessment using airborne and biological markers. The reported pathophysiological consequences of ETS exposure are examined in the context of dose and exposure. The conclusion is that it is extremely difficult, if not impossible, to extrapolate from active smoking to ETS exposure with any degree of reliability.

Key words: Environmental tobacco smoke, nicotine, cotinine, adducts, cancer, risk assessment, pathophysiology

Introduction

Tobacco smoke is an exceedingly complex matrix, consisting of several thousand constituents. As it is dispersed in the atmosphere, its chemical and physical complexity can be increased through reactions among its constituents and through evaporation, condensation, coagulation and adsorption or impaction on surfaces [1]. Tobacco smoke as it exists in the ambient environment is termed environmental tobacco smoke (ETS) and is clearly a complex and dynamic material whose properties are influenced by numerous factors. With recent concern that exposure to ETS may present a health hazard to the non-smoker [2,3], a number of risk assessments have been published dealing principally with the possible relationship of ETS to mortality and lung cancer [4]. Among several approaches used for ETS risk assessment has been the comparison between exposure to ETS and active smoking [5–8]. Inherent in such an approach is the assumption that ETS is a dilute form of mainstream smoke (MS) inhaled during active smoking, and that other than the differences in concentration, exposure conditions are similar. Considerable information exists concerning the properties of MS and the conditions of exposure during active smoking [9,10], in large part because material can be collected under reproducible conditions that simulate those to which the smoker is exposed. In contrast, the

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dynamic nature of ETS precludes its characterisation and assessment of exposure to a degree of accuracy possible with mainstream smoke.

Certain criteria must be considered in conducting a risk assessment of a material [11]. Three of these involve consideration of composition and exposure:

1. *A hazard identification*, in which it is determined whether a particular substance is causally linked to specific health effects.
2. *An exposure assessment*, in which the extent humans are exposed to the material has been determined.
3. *A dose-response assessment*, in which the amount of exposure to the material and the probability of occurrence of the specific health effects have been determined. It is the purpose of this article to examine the question of whether exposure to ETS can be assessed by extrapolation from active smoking.

Characterisation of ETS

The following discussion will be concerned with cigarettes only. Mainstream smoke (MS) is that smoke drawn into the mouth through the butt end of the cigarette by the active smoker. Sidestream smoke (SS) is defined as all other smoke emitted from the cigarette with the vast majority being the smoke released from the burning end of the cigarette between puffs [12]. In addition to MS, the active smoker is exposed to SS at levels higher than the non-smoker because of the proximity to its generation. ETS is composed of both SS and exhaled mainstream smoke (EMS), the material exhaled by the active smoker. While it is generally accepted that SS makes a larger contribution to ETS than does EMS, the relative contributions of each material to ETS have not been systematically examined. There is some evidence that EMS contributes little to the gas phase of ETS, however, it does contribute significantly to the particulate phase of ETS [12]. With certain tobaccos, EMS may contribute over 40% of the particles of ETS.

The properties of ETS are influenced significantly by a number of considerations including type of tobacco smoked and smoke density, as well as environmental factors such as dilution, ventilation, temperature, humidity, lighting and adsorption onto surfaces. Additionally, chemical reactions occur changing the composition of ETS; e.g. with time after generation, nitric oxide is converted to nitrogen dioxide [12]. The changes that occur as ETS lingers indoors are termed aging, and contribute significantly to the complexity and dynamic nature of ETS. Because of these factors, it is impossible to provide a definitive chemical and physical description of ETS, its character differing depending on conditions that exist at any given time. As a result, little consistent information exists on the characteristics of ETS under ambient conditions in indoor environments that would allow generalisations about its composition to be made. Because the frequency of puffing and the depth of inhalation differ among smokers, it should be apparent that the relative contributions of SS and EMS to ETS will be different for each ambient environment. Therefore, in addition to environmental factors described previously, the chemical and physical properties of ETS are dependent upon the smoking patterns that occur in an indoor environment. The origins and properties of ETS have been reviewed in detail elsewhere [12].

These considerations notwithstanding, numerous studies have been conducted in an attempt to characterise ETS. These have included the analysis of freshly generated SS, SS allowed to age in controlled environmental chambers, SS allowed to age in well-controlled experimental indoor environments, and ETS in a number of typical indoor environments. Each of these situations has specific limitations as to its usefulness in characterising ETS.

Considerable effort has been directed at characterising freshly generated SS as

a surrogate for ETS, and much data exist on the chemical composition of this material [13–19]. Serious problems are inherent in utilising this approach. First, and most importantly, ETS is much more complex and variable than SS generated in the laboratory due to the presence of undefined proportions of both SS and EMS and the influence of aging on ETS components. Secondly, SS is produced under conditions that do not necessarily represent the smoking pattern of individuals. SS is generated under standardised smoking conditions adopted over 20 years ago in apparatuses that allows it to be rapidly collected for analysis. The conditions are almost always one puff/min of 2 sec duration and a volume of 35ml. Since people smoke with different patterns these conditions do not necessarily simulate those of most smokers [20], and as a result, the quantities of materials released into ambient air will likely vary from those generated using smoking machines. The same objections about standardised smoking conditions could be raised regarding the composition of MS, as well.

The environmental conditions present during generation will influence the levels of chemicals in SS. This is illustrated by the effect of the velocity of air passed over the tip of the burning cigarette when generating SS [21]. In this study, the level of dimethylnitrosamine in SS varied as a function of air flow. Flow rates of 250, 500, 1000, and 1500ml of air/min yielded levels of dimethylnitrosamine of 90, 250, 530, and 680ng/cigarette, respectively. Therefore, depending on the conditions used for generation and collection, values for SS may vary greatly. This is illustrated by the wide range of values reported in the literature for nearly one hundred chemicals reported to be present in SS [15].

Compared to the study of freshly generated SS, utilisation of environmental chambers offers the opportunity to examine the properties under controlled, although not necessarily realistic conditions. The most extensive examination of SS-derived ETS under these conditions appears to have been performed by Eatough and colleagues [14,15,22]. They have utilised an unventilated teflon chamber in studying the properties of ETS originating from SS generated within the chamber. Use of the teflon chamber permits ETS to be studied in a setting where results are not influenced by ventilation or surface properties. Under these conditions, a comprehensive analysis of the chemical composition of the gas and particle phases of ETS was performed, and the behaviour of the particulate phase examined. For example, it was observed that nitrogen dioxide was the major inorganic acid present in the gas phase of ETS, and nicotine, 3-ethenylpyridine, and pyridine were the principal nitrogen bases present. Major particulate phase organic compounds were nicotine, mysomine, solanesol, nicotyrine and cotinine. Greater than 95% of the nicotine was present in the gas phase. As the ETS aged, particles underwent at least three changes. Particles deposited on the wall of the chamber, they coagulated increasing in size, and evaporation from the particles was also significant. The effect of UV radiation was also examined, and it was noted that the level of gas phase nicotine decreased with a concomitant, but less than stoichiometric increase in particulate phase nicotine. An important class of compounds, the nitrosamines were not examined in this system. It will be of considerable interest when the levels and behaviour of the volatile and tobacco-specific nitrosamines are examined under such controlled conditions.

Using a stirred stainless steel chamber to study the properties of ETS, it was reported that smoke particles underwent evaporation over the first few hours [23]. As the ETS aged, particle size increased due to a combination of coagulation and removal of smaller particles by deposition on the surface of the chamber. Similar observations have been made using a ventilated steel chamber [24].

The decay of a number of SS-derived components has also been studied in a

non-ventilated glass and stainless steel chamber [25]. In particular, polycyclic aromatic hydrocarbons (PAHs) decay at different rates during aging depending on their molecular weights; PAHs below 156 daltons had a longer half-life than those above this value. As with other experimental systems, nicotine decayed more rapidly than particulate material.

Studies have been reported using a modified trailer in which conditions can be controlled with respect to ventilation, temperature, humidity, and circulation [15,22]. Such an environment can be made to simulate closely ambient indoor conditions. Some important observations were made concerning the behaviour of SS-derived ETS in this setting which were similar to those of other workers [12]. For example, the absolute decay of various constituents of ETS was primarily controlled by the rate of ventilation. The rate of decay of nicotine was the most rapid of the components studied, while the NOx–NO species were the most stable.

While controlled chamber studies have provided useful information about ETS, the results must be interpreted with a degree of caution. These conditions only partially simulate the ambient environment in which non-smokers are exposed to ETS. For example, no studies have examined the behaviour of ETS when persons are present in the chamber or when ETS has been generated by smokers so that EMS is also present. In an effort to obtain realistic data on ETS exposure, numerous studies have examined selected chemicals and particles in ETS under a variety of ambient conditions [26–32].

Problems exist in the interpretation of these data, as well. In general, only a few substances have been investigated in each study, with sampling performed over single periods of relatively short time (24 h or less). Such a sampling protocol will fail to describe the daily variations in ETS levels that exist in indoor environments as well as fail to provide a measure of chronic exposure. The lack of specificity of most of the measured substances for ETS (e.g. carbon monoxide and respirable suspended particles) limits conclusions that can be drawn about the composition of ETS in these studies.

Exposure assessment methods and interpretations

It should be clear that there is no defined, reproducibly characterised entity known as ETS, rather it is a constantly changing substance influenced by numerous environmental and personal factors. At present, the published research represents little more than a broad representation of the nature of ETS. Therefore, it is currently not posssible to compare the risks, if any, of exposure to ETS with those reportedly associated with active smoking based on the chemical compositions of each of these materials.

As an alternative solution to the problem of characterising ETS, several approaches have been utilised to assess ETS exposure with the goal of predicting possible related health effects. These efforts have involved the assessment of exposure by use of questionnaires, modelling, surrogate airborne markers, and the assessment of internal dose by use of biological markers (biomarkers).

Reliance on questionnaires alone to assess exposure is fraught with numerous problems including lack of standardisation and validation, responder bias and potential misclassification of subjects. At best their use represents an indirect measure of exposure and cannot provide any quantitative information on specific or total exposure levels or doses of biologically relevant chemicals at target sites [33–36]. Questionnaires can have value when used as part of a more comprehensive exposure assessment. For example, an index of exposure has been developed, which includes questionnaires as one component, along with a daily diary, that correlates well with nicotine collected by a personal monitor [37].

Modelling has been used to assess concentrations of ETS constituents and to estimate exposures [5,6,38]. Data from other studies are normally used in the modelling and, additionally, this approach

requires assumptions which generalise and often oversimplify the exposure conditions.

The use of airborne markers and biomarkers offer the best opportunity to assess exposure to ETS. Unfortunately, reliance on either of these assessments alone for such a complex and dynamic mixture as ETS may result in misleading information. For example, the external dose may not be related to the internal dose as absorption, distribution metabolism and elimination may differ among individual components (particles, water-soluble chemicals, organic materials). The presence of a biomarker in a non-target tissue does not necessarily correlate with the level of a potentially toxic species at the critical cellular site nor whether a disease will result. These limitations in the use of airborne and biological markers are present when applied to exposure assessment for ETS.

Assessment of external exposure

Due to the complex chemical and physical nature of ETS, investigators have relied on tracers, or surrogates, in measuring external exposure to ETS. The National Research Council [2] has provided criteria which should be satisfied in using a surrogate for ETS:

1. It should be unique or nearly unique to ETS.
2. It should be present in sufficient quantity that concentrations can be easily detected in air, even at low smoking rates.
3. It should be characterised by similar emission rates for a variety of tobacco products.
4. It should be in a fairly constant ratio to the components of interest under a range of environmental conditions encountered and for a variety of tobacco products.

Unless the first criterion is fulfilled, the remaining criteria are of less significance. To date, no single material has satisfied these criteria.

Respirable suspended particles (RSP) and nicotine have been used most frequently as surrogates for ETS. The use of RSP fails to satisfy the first criterion because of its lack of specificity to ETS. There is a significant level of background RSP not related to ETS in the indoor environment. This has been demonstrated using the property of ultraviolet absorption of RSP as representative of the ETS-specific portion of RSP [29,30]. In several environments where smoking was permitted, it was found that ETS contributed less than 40% of the particles in the indoor environment. If RSP in an indoor environment is to be attributed to ETS, it is necessary to rule out all other sources of RSP. This has not been done satisfactorily in the studies reported to date.

While the measurement of RSP may serve as an index of exposure, it is not a measure of the dose or the amount of particulate material that will be retained in the lungs of those exposed. It is the amount of material retained in the lungs that is believed to have a relationship to health effects, not the amount to which a person is exposed. In fact, the relative retention of ETS particles has never been measured.

Different deposition patterns are to be expected for the particles in ETS and those in MS because of the different breathing patterns of the two population groups. An active smoker inhales MS by mouth often with a deep inhalation followed by a prolonged respiratory pause. Such a manoeuvre increases residence time of particles and gases in the entire respiratory tract, optimising conditions for deposition. In contrast, a non-smoker would inhale ETS principally through the nose using a regular breathing pattern which is much more shallow than that used by active smokers. The shallow breathing pattern would reduce the degree of pulmonary deposition of particulate materials of ETS in non-smokers compared to MS in the active smoker.

Risk assessments for lung cancer have been performed using estimated exposure to RSP from ETS [6,39]. The values used in these calculations were dependent on a number of assumptions that did not consider the limitations of using RSP as a surrogate for ETS. Consequently, the risk values are open to question.

Nicotine has been measured in ambient air using area sampling [40–42] and with personal samplers [37,43,44]. Personal samplers monitor the immediate environment of the subject permitting a more accurate assessment of personal exposure than occurs with area sampling. While airborne nicotine would be specific for ETS, problems exist in using it as a surrogate. Nicotine in ETS is principally in the gas phase [15], while nicotine in MS is almost exclusively in the particulate phase. Therefore, in ETS, nicotine would be serving as a surrogate for gas-phase components only.

Additionally, nicotine in ETS decays more rapidly than other gas-phase components [22], in large part due to its adsorption onto surfaces. It is likely that, once smoking has stopped in a room, the adsorbed nicotine will be slowly released back into the atmosphere. If this occurs, a low level of airborne nicotine may be present in an area where smoking had not occurred for some time giving an inaccurate representation of total ETS exposure.

The ratio of RSP/nicotine has been discussed as a possible monitor for ETS in ambient environments, and in particular as a means for quantifying the ETS-specific RSP [45]. Laboratory studies have given an average ratio of 13:4. Using values for RSP and nicotine from field surveys, it has been concluded that the relationship between these two materials is too variable to use for predictive purposes [46].

The mutagenic properties of RSP have been used to assess exposure to ETS [26]. The principal problem with this approach is the interpretation of the results. The significance of the presence of airborne mutagens has not been established nor have quantitative measures of retention of mutagenic materials been obtained. Because of these uncertainties, the measurement of airborne mutagenicity has provided little information in assessing exposure to ETS.

To date no single material satisfies the criteria as a marker for ETS. Consequently, it has not been possible quantitatively to assess the external dose of ETS a non-smoker receives.

Assessment of internal dose

As an assessment of exposure to ETS, biomarkers can serve as surrogates for the internal dose received. Criteria have been proposed that an effective marker should satisfy [47]:

1. It should be tobacco-specific in order to be certain of its origin.
2. It should have a long half-life so that it serves as an index of exposure over an extended period of time.
3. The marker should give a valid indication of the health risks of exposure.
4. Analytical techniques should be available that can reliably and conveniently measure the low levels of the marker present in non-smokers exposed to ETS.

Biomarkers of ETS exposure have been measured in biological fluids of humans. Several biomarkers have been utilised with varying degrees of success in the assessment of exposure to ETS, including nicotine and cotinine in saliva, blood, and urine, DNA and protein adducts in blood, and mutagenic activity in urine. From these results, investigators have drawn conclusions about exposure, risk of disease, and mortality.

When interpreting studies in which biomarkers have been used to assess exposure or risk, a number of factors must be considered [48]. Data on variation among individuals in absorption, metabolism (including bioactivation and detoxication), kinetics, distribution, excretion, binding to macromolecules and cellular repair must be evaluated. In the

use of biomarkers for assessment of exposure to ETS and assessment of potential health risks, such considerations have not been employed consistently.

Biomarkers such as nicotine, or one of its metabolites, cotinine, in body fluids have been used to assess internal exposure to ETS [41,48–50]. In general, salivary and urinary cotinine provide the best relationship with self-reported exposure to ETS [47,51]. Levels of cotinine in body fluids tend to correlate directly with the number of smokers in the household, the number of hours of exposure, the number of smokers among acquaintances, and are higher in non-smokers married to smokers than in those married to non-smokers.

Nevertheless, significant limitations exist in the use of nicotine or cotinine to assess exposure to ETS. At best, levels of nicotine or cotinine are useful qualitatively to assess exposure. Too many limitations exist for them to be considered quantitative dosimeters from which risk can be estimated [52]. In virtually all studies reported, single samples are taken in the assessment of exposure. Such values are an index of exposure at a specific point in time and do not represent the cumulative exposure that would be required properly to evaluate exposure to ETS. Importantly, the vast majority of nicotine in ETS is in the gas phase while nicotine in MS is predominantly in the particulate phase [52,53]. Therefore, values for nicotine or cotinine in body fluids represent the inhalation of physically different materials in the two exposure groups making their comparative use questionable. Additionally, gas-phase nicotine and particulate-phase nicotine decay at different rates under experimental conditions [22]. Levels of nicotine or cotinine in body fluids provide no information on exposure to other chemicals, particularly those in the particulate phase which are believed to have the most relevance to potential adverse health effects.

It was once thought that one of the attractive features of using nicotine and cotinine as biomarkers was their tobacco specificity. Recent studies indicate that nicotine is not unique to tobacco. A number of vegetables in our diet have been shown to contain nicotine [54,55]. The fact that nicotine, and consequently cotinine, can arise from non-tobacco sources complicates the interpretation of the low-level values of these chemicals that are measured in the body fluids of non-smokers.

Cotinine is only one of a number of metabolites of nicotine and evidence is now indicating that nicotine-n-oxides or trans 3'-OH-cotinine, rather than cotinine, may be the most abundant metabolites of nicotine in the urine [56–58]. Cholerton *et al.*, [56] report a larger coefficient of variation for cotinine than other nicotine-derived metabolites in the urine of smokers. Variations in the metabolic formation of cotinine among non-smokers would further confound the interpretation of cotinine levels.

Complicating this problem even further are pharmacokinetic factors. Nicotine appears to be metabolised at different rates in smokers and non-smokers [59–61]. The half-life of nicotine in plasma appears to be shorter for smokers than for non-smokers; therefore, the relative relationship of values between the two groups will differ depending upon the time of sampling.

Both intralaboratory and interlaboratory variations have been reported for urinary cotinine values [62,63], indicating that comparisons of values among laboratories should be made with caution. Such methodological considerations are of particular significance when values are low as is the case with exposure to ETS.

It seems evident that the measurement of cotinine in body fluids will likely provide misleading information regarding the quantitative exposure to ETS. Considering the factors discussed, a compelling argument can be made against using nicotine or cotinine values for either a quantitative comparison of exposure between smokers and those exposed to ETS or in an

attempt to assess the possible risk of exposure to ETS.

Urinary cotinine as a predictor of health risks of exposure to ETS should be used with caution [64]. Nevertheless, a risk assessment estimating ETS-related mortality has been made using such values. One study reported that urinary nicotine values in non-smokers were 0.7% of the level found in smokers [8] and the assumption made that there are premature deaths from the inhalation of ETS which may be approximately 0.7% of that due to active smoking resulting in 1,000 deaths a year in Great Britain and 4,000 deaths a year in the United States. No consideration was given to the limitations in the use of this marker. Additionally, the authors assumed that the relationship of dose-to-risk is linear between these two exposure extremes, an assumption that has not been shown to be valid. Clearly, this risk assessment is overly simplistic and confounded by a number of significant conceptual problems.

Wigle *et al.* [65], used values for urinary cotinine of active smokers and non-smokers exposed to ETS to assess the relative exposures of non-smokers to components of tobacco smoke that have been reported to be toxic. They concluded that persons exposed to ETS for 20 or more hours per week have exposures to six compounds that have been designated as known or probable human carcinogens which are at least 2% of those of active smokers and, for certain of these compounds, may be more than 20%. In arriving at these estimates, the authors made a number of assumptions which ignore the complexity of the exposure situation. In particular, SS was used as a surrogate for ETS. Such a premise is clearly inappropriate and invalidates any quantitative relationships that might be developed.

DNA and protein adducts have been utilised as biomarkers to assess internal exposure to ETS. Adducts are products derived from covalent reactions between chemicals and biological material such as DNA and proteins. The formation of DNA adducts is reported to be associated with mutagenesis and carcinogenesis [66,67], and adducts are viewed as markers of the biologically effective dose of carcinogens in humans. Recent evaluation of the role of adducts in carcinogenesis indicates that the relationship may not be as direct as initially thought [68].

In spite of the lack of correlation between adduct levels and cancer in a number of studies [69–72] considerable interest continues in their use as molecular dosimeters for carcinogenesis. Although studies have started to examine their possible role in the assessment of exposure to ETS, little useful information currently exists in this context.

For many chemicals, adduct formation following metabolic activation is a necessary, but not sufficient, event to initiate carcinogenesis [73,74]. The formation of adducts may not occur on a region of the genome that is critical in the carcinogenic process. The role of DNA repair must also be considered [75,76]. The variability in repair capabilities in humans [77] will influence the level of adducts present in a tissue. Additionally, genetic polymorphism of drug metabolism in humans has been shown to result in wide inter-individual capacities to activate carcinogens metabolically [78]. Because cancer is a multistage process, and because the level of adducts may be influenced by a multitude of factors including diet [79] it is thought to be unlikely that DNA adducts will provide precise quantitative dosimetry for predicting cancer risk [80], particularly where the level of adducts is as low as observed for ETS exposure. Another line of research in this area involves proteins as target molecules for adduct formation with the goal of serving as a surrogate for DNA adducts [81]. Because of its abundance, haemoglobin has been used to monitor adduct levels associated with exposure to tobacco smoke [82,83].

A potentially attractive aspect of the use of adducts as a dosimeter for ETS exposure, is that they may be useful in

monitoring exposure, at least qualitatively, on a more chronic basis than with other markers. To date, no tobacco-specific adduct has been identified that is capable of fulfilling this goal. Adducts of 4-aminobiphenyl-haemoglobin (4-ABP-Hb) and of benzo[a]pyrene diol epoxide-1-DNA (BPDE-1-DNA) in white blood cells have been compared in smokers and non-smokers [82–84]. While both 4-ABP and benzo[a]pyrene (BP) have been classified as carcinogenic, neither is tobacco-specific. Levels of 4-ABP-Hb adducts have, however, been used to distinguish smokers from non-smokers. The levels of 4-ABP-Hb adducts in non-smokers have been reported to be about one fifth the level found in smokers [82,83]. In one study, BPDE-1-DNA adducts were of little value in distinguishing the two groups [83]. Over a 48 h period, there was little consistency in the presence of adducts in smokers, with many smokers having no detectable levels. Additionally, there was no apparent correlation between the level of 4-ABP-Hb adducts and the level of BPDE-1-DNA adducts in either group.

Adducts of 4-ABP-Hb and 3-ABP-Hb have been measured in the blood of non-smokers with varying degrees of exposure to ETS as assessed by the presence or absence of detectable serum cotinine [82]. In non-smokers exposed to ETS, the 4-ABP-Hb levels were about 40-fold higher than the level of 3-ABP-Hb which in many subjects was below the limit of detection. Due to the lack of a clear cut effect of ETS exposure on 4-ABP-Hb adduct levels, and the inconsistent detectability of 3-ABP-Hb adducts, the usefulness of these markers to discriminate non-smokers exposed to ETS from those who are not exposed, appears questionable.

Recent studies indicate that the turnover of adducts may be more rapid than originally thought, limiting their usefulness to monitor chronic exposure. While the lifespan of haemoglobin is 120 days [81], levels of 4-ABP-Hb adducts in smokers returned to background levels in 6–8 weeks following cessation of smoking [82]. NNK is a tobacco-specific nitrosation product of nicotine that is present in MS and SS and has been classified as carcinogenic in animals [85]. Removal of adducts induced by the injection of NNK, has been examined in rats [69]. Rates of removal of different adducts in target tissues was variable and rapid, occurring within several days. These data indicate that NNK-induced adducts may not be useful as a dosimeter for tobacco smoke exposure.

A very sensitive method for examining the presence of adducts is ^{32}P post-labelling. This technique provides a semi-quantitative estimate of the adduct level in a tissue. At present, there has been little application of this technique in assessing exposure to ETS. In spite of its sensitivity, there are limitations associated with the ^{32}P post-labelling technique. It does not allow identification of the adduct, and basal levels of adducts are reported to increase with age, at least in animals [86]. Using this technique, no increase in DNA adducts was reported in monocytes of non-smokers heavily exposed to ETS [87].

In order to compare the potential risks of exposure to ETS with those reported for active smoking, an extrapolation from high-dose exposure to low-dose exposure is required. DNA adducts have been proposed as a means to do this. The relationship between external dose and biological dose, as assessed by DNA adducts, is dependent on the absorption, distribution, metabolism and excretion of the chemical of interest. The interpretation of biological dose using DNA adducts is influenced by several factors, including the location of the adducts on the genome as well as the mutagenic efficiency of the material, including the base that is modified and the effectiveness of the repair process. Additionally, for certain chemicals, the level of adduct formation is not linearly related to the dose administered. From the existing data, no absolute information is available relating the presence of adducts to a quantitative or qualitative assessment of exposure to ETS.

As a measure of exposure to ETS, studies have been conducted on the capability of concentrated extracts from the urine of non-smokers and persons exposed to ETS to induce mutations in bacteria [88–94]. The rationale behind this approach is that the presence of mutagens in the urine may be an indication that the person has been exposed to chemicals that can ultimately induce cancer. Compared to the mutagenicity of the urine of smokers, activity in the urine of those exposed to ETS is quite low, variable and not always above background levels.

A number of problems exist with the studies attempting to relate urinary mutagenicity to ETS exposure. The experimental conditions of exposure to ETS have often been unrealistic in comparison to that occurring in the ambient environment. Methodological differences exist among studies possibly contributing to some of the inconsistencies. The studies have not always been controlled for the presence of dietary mutagens, an important confounding factor [95]. Importantly, the putative mutagens have not been identified. Finally, the biological significance of low-level mutagenicity in urinary concentrates has not been established. Due to these factors there is little reason, at present, to believe that urinary mutagenicity can be used to assess exposure to ETS or to assess risk to cancer [93].

Extrapolation models

An important consideration in the dose-response analysis of risk assessment is the extrapolation model used at the low-dose end of the curve. Traditionally, the linear non-threshold dose-response model has been used in the quantitative risk assessment of carcinogens. Current evidence brings this concept into question [96] and necessitates a rethinking of this process. The theory presented is that, at low doses, initiation may occur but unless exposure to high doses of promoters then occurs, tumours will not develop. This line of reasoning has considerable impact on the procedures used for analysing low-dose exposure as it relates to extrapolation of cancer risk from active smoking to exposure to ETS.

In spite of the distinct differences in dose received from active smoking and exposure to ETS, the extent of exposure to ETS and active smoking has been compared through the use of cigarette equivalents [21,40,43,46,47,65,97]. This approach attempts to convert exposure to ETS into an equivalent exposure from active smoking with the assumption that the risk from ETS exposure is proportionally comparable to the risk from active smoking. This procedure is an oversimplification of the exposure conditions and will provide potentially misleading information [3].

Pathophysiological consequences and implications

As indicated above, it is extremely difficult to extrapolate from active smoking to ETS exposure with any degree of reliability. Similarly, the data do not point to consistent evidence of pathophysiological consequences of ETS based on exposure and dose. Some examples will be presented to illustrate this point.

Several studies have reported that, functionally, smokers may have reduced ventilatory function at rest and a reduced exercise capacity with a greater oxygen debt accumulation [98–101]. For ETS-exposed non-smokers, the effects on ventilatory function and exercise capacity reductions are not consistent. While a few studies show some functional impairment, the majority do not. First of all, it is difficult to determine if the test situation mimics real-life exposure. The conditions to which subjects are exposed are often not relevant to ETS exposure. One study where subjects were passively exposed to cigarette smoke illustrates this point [102]. After drawing the puff through the apparatus consisting of a solenoid, capacity vessel and pump, the MS was discharged into the test room along with the SS. Therefore, the subjects were essentially breathing diluted quantities of the same constituents as an active smoker. The exposure conditions were also rather

extreme. Initial concentrations of particulate matter were >4mg/m^3 and carbon monoxide levels were 24 ppm. After 2 h, the particulate concentration dropped to only 2mg/m^3. Therefore, these conditions are not representative of ambient ETS exposure.

Even in this study [102], no change was found in the FEV_1 of the subjects at rest. When bicycle exercise was performed, the only change found was a slight increase in heart rate at two to five time points that was statistically significant but not biologically important.

Another problem in trying to identify possible effects of ETS on pulmonary function is the inaccurate or broad ranges of exposure as represented in either the ETS-exposed or -unexposed groups. Usual confirmation of ETS exposure or lack of active smoking is through questionnaires without chemical confirmation. No matter how limited chemical confirmation techniques are, questionnaires are less reliable. Most epidemiological studies involve spousal exposure and ignore whether smoking occurs in the home to any significant degree or whether spousal exposure is compounded by workplace or social exposure. Intuitively, it might be expected that smokers socialise with others who also smoke more often than do non-smokers. The other major consideration may be tied to the general health status or awareness of smoker households compared to non-smoker households. It would seem very important to match groups for diet and exercise as well as other health indicators.

Functional studies

In contrast to the studies reported on MS, it would appear that there is little agreement among studies as to the effects of ETS on pulmonary parameters. Even within studies, unexplainable peculiarities appear that raise questions of reliability. Certain age groups of particular populations are found to be affected where other population segments in the same study show increased pulmonary function capability. In a comprehensive review of this subject the results of studies were regarded as being too variable to permit a conclusion concerning long-term ETS exposure and possible impaired respiratory health or pulmonary function in non-smoking adults [103].

Studies typically are further complicated by the possibility of suggestibility. Suggestibility is the reverse of the placebo effect. These studies are performed to determine the magnitude of the psychosomatic effect and hope to answer the question: "If the subject expects an adverse effect to occur, will this be reflected in a measurable response?" Here again, there is no good agreement. One study reports a 50% increase in airways resistance following a positive suggestion that the subject would be breathing a substance that may be irritating and make it harder to breath [104]. In another study, subjects who could easily tell whether or not they were breathing the smoke, were exercised at a level to increase minute ventilation to about 2.5 times resting ventilation. These subjects showed a dose-related response to sham or zero smoke, and two levels of ETS exposure [105]. The magnitude of change in pulmonary function parameters was minor in most cases and of no physiological significance. The experiment was flawed by the failure clearly to separate the pyschological influence from the physiological effects and to establish any real controls, whereas the previously cited study [104] unquestionably separated the two components. Furthermore, in this study [105] it appeared that all smoke, including the MS generated by the smoking machines, was presented to the subjects.

The question of allergic response to tobacco smoke has been raised frequently, and was investigated by McDougall and Gleich [106], who reported that tobacco and tobacco smoke allergies were not demonstrable. It might thus be concluded that most of the apparent irritation in the presence of ETS is psychologically based.

When considering asthmatic patients, where active smoking has sometimes

been reported to be capable of triggering attacks, the evidence is not well established for ETS. Pulmonary function tests of asthmatics produced no change in expiratory flow rates. However methacholine challenge did produce a slight but significant increase in airway reactivity [107]. Other investigators studied the effects of ETS on asthmatics and found variable and inconclusive results in pulmonary function, but again found the increased reactivity to challenge; this time to histamine [108]. The results seem reasonable; however the regimen was not clearly stated. The mixing of MS and ETS may be a confounding problem of this study, as well. In summary, these results suggest a highly variable functional response to ETS even under laboratory conditions.

Cancer types, locations and frequencies

Use of tobacco products has been reported to be associated with cancers of various types and in various organ systems depending upon the tobacco product used. A review which addresses the comparisons between active smoking and exposure to ETS, concludes that more research needs to be done to demonstrate a strong association between ETS and cancer in the non-smoking population [109].

These authors begin with the hypothesis that the association between ETS and lung cancer must be possible based on the evidence from active smoking. They then examine the criteria set forth in the Surgeon General's report of 1964, and cite the inconsistencies in the results of both prospective and case-control studies. They make a specific point of the necessity for carefully documenting tumours using good histopathological techniques. In their own previously reported and unreported studies, they found that there is a preponderance of Kreyberg type I class tumours associated with smoking. In never-smokers, the preponderance of tumours are classified as Kreyberg type II. Within these catogories the squamous cell type (type I) was predominant in smokers, "with lesser but significant causative effect on the glandular type". In non-smokers, the predominant type is the glandular adenocarcinoma type II tumour. Other authors [110–111] suggest that ETS is limited to squamous cell types of tumours. If this is the case, the numbers of tumours potentially attributable to ETS would be very small considering the low incidence of this type of lung cancer in non-smokers. There is some support for squamous cell tumours being the most likely to be caused by ETS [112], quoted by the US Surgeon General [113]. In a closely monitored study in Olmsted County, Minnesota, Beard and his colleagues found that the incidence rate for squamous cell tumours dropped remarkably in the 1965–1974 period, presumably as smoking decreased. Small cell tumour incidence, also associated with smoking, decreased but not as dramatically. The incidence of adenocarcinoma continued to rise. There are several conclusions that can be drawn:

1. If Dalager *et al.*, [110] and Pershagen *et al.*, [111] are correct in concluding that squamous cell and small cell tumours are the predominant types associated with both smoking and exposure to ETS, then the risk of lung cancer from ETS is very small since this tumour is rare in non-smokers.
2. Since adenocarcinoma of the lung continued to rise in the Olmsted County study and is purported by some investigators to be the predominant type for ETS exposure, the association between ETS and adenocarcinoma is incorrect, meaning that some other cause is associated with the development of adenocarcinoma of the lung.
3. ETS may not, in fact, cause cancer of the lung at all, or if it does, perhaps it is associated with several types of tumours but not at a very high level.

Regardless of who is correct, more careful documentation is necessary of the histological types and incidence of lung tumours in order to determine an accurate and meaningful risk.

Conclusions

Since ETS has not been adequately characterised, there are insufficient data on which to base a hazard analysis. Accordingly, there are not enough data available on which to base an exposure assessment for ETS. Due to the dynamic nature of ETS, it is impossible to relate ETS to MS chemically or physically. In the absence of this relationship, it is inappropriate to make any extrapolations from what is reported about the effects of active smoking to possible effects of exposure to ETS. Therefore, any calculation of risk from exposure to ETS based on extrapolations from calculated risks of active smoking is, at best, not reliable and, most probably, of no value whatsoever. It is important, therefore, to consider ETS as a distinct entity, and further research is needed to test hypotheses based on valid protocols that meet the criteria established for the epidemiology of weak associations.

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